

Metabolic Risk Factors Are Associated With Non-Hepatitis B Non-Hepatitis C Hepatocellular Carcinoma in Taiwan, an Endemic Area of Chronic Hepatitis B

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Metabolic risk factors, such as obesity, fatty liver, high lipidemia, and diabetes mellitus are associated with increased risk for nonviral hepatocellular carcinoma (HCC); however, few nonviral HCC studies have stratified patients according to underlying etiologies. From 2005 to 2011, 3,843 patients with HCC were recruited into the Taiwan Liver Cancer Network. Of these patients, 411 (10.69%) who were negative for hepatitis B virus (HBV), surface antigen, HBV DNA, and anti-hepatitis C virus (HCV) antibody were classified as non-HBV non-HCV (NBNC)-HCC. Detailed clinical analyses of these patients were compared with age- and sex-matched patients with HBV-HCC or HCV-HCC for the associated metabolic risk factors. For this comparison, 420 patients with HBV-HCC and 420 patients with HCV-HCC were selected from the 3,843 patients with HCC. Multivariate analyses showed fatty liver (by echography), high triglyceride levels (>160 mg/dL), and diabetes mellitus history to be significantly associated only with NBNC-HCC and not with the matched patients with HBV- or HCV-HCC. When the patients with HCC were further divided into four groups based on history of alcoholism and cirrhotic status, the group without alcoholism and without cirrhosis exhibited the strongest association with the metabolic risk factors. Based on trend analyses, patients with NBNC-HCC with or without alcoholism were significantly different from the matched patients with HBV- or HCV-HCC, except for patients with alcoholism and cirrhosis, in having more than two of the above three risk factors. *Conclusion:* Metabolic risk factors are significantly associated with nonviral HCC, especially for patients without alcoholism in Taiwan. Because the prevalence of viral HCC is decreasing due to the success of universal vaccination and antiviral therapy, strategies for cancer prevention, prediction, and surveillance for HCC will require modification. (*Hepatology Communications* 2018;2:747-759)

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide.^(1,2) Major risk factors for HCC include chronic viral hepatitis, cirrhosis, alcoholism, alpha-toxin exposure, and nonalcoholic fatty liver disease (NAFLD).⁽¹⁻⁴⁾ HCC incidence varies geographically. In countries where liver cirrhosis is mainly related to chronic viral hepatitis, such as Africa and

Asia, the HCC incidence is high.⁽¹⁻⁴⁾ In Western countries, nonviral etiology (such as alcoholism or NAFLD) is the main cause of liver cirrhosis and the HCC incidence is low.⁽¹⁻⁴⁾ In Taiwan, hepatitis B virus (HBV) infection is a major risk factor for HCC.⁽⁵⁻⁹⁾ As of 2015, HCC still ranked second among the leading causes of cancer-related death in men in Taiwan,⁽¹⁰⁾ and researchers have estimated that

Abbreviations: anti-HBc, hepatitis B core antibodies; BMI, body mass Index; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CHO, cholesterol; DM, diabetes mellitus; HBsAg, hepatitis B virus surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease; NBNC, non-hepatitis B non-hepatitis C; SH-HCC, steatohepatitic hepatocellular carcinoma; TG, triglyceride; TLCN, Taiwan Liver Cancer Network.

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80% of HCC cases in Taiwan are related to chronic viral hepatitis.⁽⁵⁻⁹⁾ In 1984, Taiwan became the first country in the world to establish a universal HBV vaccination program for newborns/infants.^(11,12) HBV vaccination has successfully reduced the incidence of HCC in children and adolescents.^(11,12) In addition, antiviral therapy for HBV and hepatitis C virus (HCV) has been practiced in Taiwan since 2003,⁽¹³⁻¹⁵⁾ resulting in a decrease in overall HCC incidence over the last decade. From 2002 to 2012, the average annual percentage change was -1.1%,⁽¹⁶⁾ and comparisons of the cancer registry data from 2011 and 2014 have shown that the incidence rate has continued to decrease.^(17,18) Therefore, incidence levels of chronic viral hepatitis in Taiwan are expected to decline in the long term, and accordingly the incidence levels of HBV- and HCV-related HCC are also expected to decrease.

However, with lifestyle changes and more westernized foods in Taiwan, the number of patients exhibiting fatty liver and metabolic syndrome has increased drastically.^(19,20) NAFLD and metabolic syndrome

have been identified as risk factors for HCC, especially for non-HBV non-HCV (NBNC)-HCC.^(4,21-25)

Hence, patients with nonviral HCC may become a significant HCC subgroup in Taiwan in the near future. Therefore, the risk factors and mechanism of carcinogenesis of nonviral HCC require further study.

The Taiwan Liver Cancer Network (TLCN) was established in 2005 with the support of government funding to systemically collect biosamples and clinical information of patients with HCC from five major medical centers across Taiwan (National Taiwan University Hospital, Linko Chang Gung Memorial Hospital, Taichung Veteran General Hospital, Kaohsiung Chang Gung Memorial Hospital, and Kaohsiung Veteran General Hospital). The TLCN has published a detailed viral hepatitis marker study of 3,843 patients with HCC recruited from 2005 to 2011.⁽²⁶⁾ During the recruitment, blood samples of all 3,843 patients with HCC were routinely examined for viral markers, including HBV surface antigen (HBsAg), HBV DNA, HBV genotype, and anti-HCV antibody. For

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patients who tested positive for anti-HCV antibody, examinations for HCV RNA and HCV genotype were also performed. These viral marker data were used to sort the 3,843 patients with HCC into four groups: HBV-HCC (2,153 patients, 56.02%), HCV-HCC (969 patients, 25.21%), B+C-HCC (310 patients, 8.07%), and NBNC-HCC (411 patients, 10.69%). The incidence of cirrhosis was lowest among the patients with NBNC-HCC (only 24.57%) compared with the incidence among patients from the other three groups of HCC: HBV (42.45%), HCV (52.22%), and B+C (53.55%). The category NBNC-HCC indicated that a patient tested negative for all three viral markers: HBsAg, HBV DNA (undetectable), and anti-HCV. In published reports, the diagnosis of NBNC-HCC only required negative results for both HBsAg and anti-HCV,⁽²⁷⁻³⁴⁾ and tests for serum HBV DNA were not performed. In this study, we added HBV DNA criteria (undetectable) for the diagnosis of NBNC-HCC because among the 2,153 patients with HBV-HCC and the 310 patients with B+C-HCC in our detailed viral marker study report, we identified 202 patients (8.20%) who tested negative for HBsAg but positive for HBV DNA.⁽²⁶⁾ The HBsAg-negative but HBV DNA-positive patients were considered to have occult HBV infection. Thus, the patients with NBNC-HCC noted in the published reports might have included a significant number of occult HBV cases, especially in endemic areas of HBV, such as Taiwan.^(7,8) The group of 411 patients with HCC who tested negative for all three viral markers in our published study series should have excluded most patients with occult HBV infections and is therefore reliable for further analysis on the risk factors and mechanisms associated with NBNC-HCC.

In this study, we performed detailed clinical analyses of the 411 patients with NBNC-HCC, focusing on the previously mentioned metabolic risk factors, and we compared these analyses with those of patients with HBV-HCC or HCV-HCC. Our results indicated that echography-detected fatty liver, diabetes mellitus (DM) history, and high serum triglyceride (TG) levels (>160 mg/dL) were significantly associated with NBNC-HCC, especially among patients without alcoholism. This study represents the largest scale examination to date of an HCC patient cohort in which patients were stratified according to their underlying etiologies and in which comprehensive clinical data were analyzed to determine metabolic risk factors.

Patients and Methods

PATIENTS

The TLCN published a detailed viral marker study of 3,843 patients with HCC recruited from 2005 to 2011 in the TLCN.⁽²⁶⁾ HCC was diagnosed in these patients through histopathological examination (87.3%) and radiology (12.7%). Patients were divided into four groups: HBV-HCC, HCV-HCC, B+C-HCC, and NBNC-HCC, based on the test results for serum HBsAg, HBV DNA, and anti-HCV antibody. The 411 (10.69%) patients negative for all three viral markers were grouped as NBNC-HCC. Detailed clinical and epidemiologic data of all 3,843 patients with HCC were obtained from the TLCN database to identify the clinical features significantly associated with NBNC-HCC compared with HBV-HCC and HCV-HCC. These data included smoking history, alcohol consumption, hypertension, body mass index (BMI), echography-detected fatty liver, DM history, and presence of cirrhosis. A nonsmoker was defined as an individual who had never smoked or who had smoked fewer than 100 cigarettes in his or her life. Positive alcoholic history was defined as alcohol consumption over 30 g/day for male patients and 20 g/day for female patients when converted to ethanol.⁽³⁵⁾ Cirrhosis was diagnosed mainly by a histopathological examination (82% for patients with HBV-HCC, 77% for HCV-HCC, and 74% for NBNC-HCC). Cirrhosis in the remaining patients was diagnosed according to clinical features or radiology. To reduce potential bias from age and sex ratio discrepancies among the groups, we randomly selected from the 3,843 patients 420 age- and sex-matched patients with HBV-HCC or HCV-HCC who also had paraffin tissue sections, without stratification for other clinical features.

If patients had no serum TG or cholesterol (CHO) data available within 6 months before or after enrollment, we examined serum levels of TG and CHO using frozen serum samples stored in the -80°C deep freezers of the TLCN tissue bank. TG and CHO levels have been reported to remain stable if serum samples are preserved in -80°C deep freezers.⁽³⁶⁾ The central clinical laboratory of Linko Chang-Gung Memorial Hospital performed the examinations for this study. We were unable to use the frozen serum samples to check the blood sugar or hemoglobin alcohol level because blood sugar and hemoglobin alcohol levels require special preservation methods.

HISTOPATHOLOGY OF THE NON-NEOPLASTIC LIVER TISSUE, FOCUSING ON FATTY CHANGE

Histopathology examination of the non-neoplastic liver tissue with a focus on fatty change was performed on the 294 available tissue sections of the 411 patients with NBNC-HCC as well as on tissue sections from the 420 matched patients with HBV-HCC and the 420 matched patients with HCV-HCC, all of whom had tissue sections from operation specimens. The proportion of fatty change in adjacent nontumor liver tissue was evaluated by two board-certified pathologists (Y-Y.S. and S-F.H.). Tissue sections were stained with hematoxylin and eosin, and fatty change was classified as four grades: 1) <5%, 2) 5%-33%, 3) >33% and <66%, and 4) \geq 66%.

The presence of steatohepatic features in the HCC tumor tissue was also evaluated in all patients with NBNC and matched patients with HBV- and HCV-HCC who had a tumor tissue component in the histology sections. The criteria for the diagnosis of steatohepatic HCC (SH-HCC) included: 1) steatosis (>5% tumor cells with macrovesicular fatty change); 2) ballooning or Mallory-Denk body formation is identified; 3) presence of interstitial fibrosis, and 4) presence of inflammatory infiltrate in the tumor.^(37,38)

The protocol for this study was approved by the institutional review boards of all five hospitals in the TLCN and National Health Research Institutes. All patients signed an informed consent form.

STATISTICAL ANALYSIS

We used the chi-square test and Fisher's exact test for categorical variables. The Mann-Whitney test and Kruskal-Wallis test were used for continuous variables (i.e., age). A binary logistic regression test was performed for multivariate analysis, and the Cochran-Armitage test was used in trend analysis. $P < 0.05$ was considered to indicate a significant difference when comparing groups.

Results

CLINICAL CHARACTERISTICS OF THE 411 PATIENTS WITH NBNC-HCC

Of the 411 patients with NBNC-HCC, 327 (79.56%) were male patients and 84 (20.44%) were female patients. Most clinical features showed no

significant sex-related differences; the exceptions were smoking ($P < 0.0001$) and alcoholic history ($P < 0.0001$). Only 3 women were smokers or had alcoholism. Additionally, DM history was significantly higher among female patients ($P = 0.0404$) (Supporting Table S1). No underlying genetic disorder was found in the medical records of the 411 patients with NBNC-HCC.

HISTOPATHOLOGY OF THE TUMOR IN ADJACENT NON-NEOPLASTIC LIVER TISSUE AND STEATOHEPATITIC FEATURES IN THE TUMOR

Liver tissue with a fatty change greater than 66% was found in 13 of the 294 patients with NBNC-HCC, 1 of the matched patients with HBV-HCC, and none of the matched patients with HCV-HCC. Because this group was too small for meaningful analysis, we divided fatty change into three grades for further analysis: <5%, 5%-33%, and >33%. Clinical features of the patients with NBNC-HCC associated with a degree of fatty change are listed in Table 1. Echography-detected fatty liver ($P < 0.0001$), BMI ($P = 0.0003$), DM history ($P = 0.0004$), high TG level ($P = 0.0150$), and cirrhosis ($P = 0.0016$) were all significantly correlated with higher degrees of fatty change in liver tissue. Alcoholic history was not significantly associated with fatty change in liver tissue ($P = 0.5625$).

A tumor component in the histology sections for evaluation of steatohepatic features was found in 294 patients with NBNC-HCC, 413 patients with HBV-HCC, and 402 patients with HCC-HCV. The incidence of SH-HCC was significantly higher in NBNC-HCC (26.87%, 79/294) than in HBV-HCC (14.77%, 61/413) or HCV-HCC (12.44%, 50/402) (Supporting Table S2). DM history was significantly associated with SH-HCC in all three groups of patients (Supporting Tables S3-S5). A higher degree of fatty change in the non-neoplastic liver tissue was significantly associated with SH-HCC only in patients with HBV-HCC or HCV-HCC. The number of female patients with HCC was significantly higher in the SH-HCC group in patients with HCV-HCC or NBNC-HCC. High CHO (>200 mg/dL) was not associated with SH-HCC. In contrast, a high CHO level was more common in patients with NBNC-HCC without steatohepatic features in the tumor.

TABLE 1. CORRELATION OF FATTY CHANGE OF TISSUE WITH CLINICAL FEATURES OF 294 PATIENTS WITH NBNC-HCC

Variables	Degree of Fatty Change in Tumor Adjacent to Benign Liver Tissue			P*
	<5%	5%-33%	>33%	
Total patients	180 (61.22%)	66 (22.45%)	48 (16.33%)	
Age				0.6670
Median(min-max)	66 (25-86)	67 (28-84)	68.5 (13-84)	
Sex				0.6968
Male	145 (80.56%)	52 (78.79%)	36 (75%)	
Female	35 (19.44%)	14 (21.21%)	12 (25%)	
Smoking [†]				0.7610
Yes/Ever	96 (55.17%)	37 (56.92%)	22 (50%)	
No	78 (44.83%)	28 (43.08%)	22 (50%)	
Alcoholic history [‡]				0.5625
Yes	53 (29.61%)	15 (22.73%)	13 (27.08%)	
No	126 (70.39%)	51 (77.27%)	35 (72.92%)	
Cirrhosis [§]				0.0016
Yes	26 (14.53%)	19 (28.79%)	17 (35.42%)	
No	153 (85.47%)	47 (71.21%)	31 (64.58%)	
Fatty liver (by echography)				<0.0001
Yes	30 (16.67%)	28 (42.42%)	19 (39.58%)	
No	150 (83.33%)	38 (57.58%)	29 (60.42%)	
BMI level				0.0003
Median(min-max)	23.4 (15.7-40.0)	25.0 (18.0-37.0)	26.4 (16.3-42.74)	
>24,	75 (43.1%)	43 (65.15%)	31 (70.45%)	
≤24	99 (56.9%)	23 (34.85%)	13 (29.55%)	
Hypertension [¶]				0.1310
Yes	52 (29.89%)	15 (22.73%)	18 (40.91%)	
No	122 (70.11%)	51 (77.27%)	26 (59.09%)	
DM history [#]				0.0004
Yes	45 (25.86%)	24 (36.92%)	25 (56.82%)	
No	129 (74.14%)	41 (63.08%)	19 (43.18%)	
Cholesterol(mg/dL)**				0.8020
>200	26 (14.53%)	12 (18.18%)	7 (14.58%)	
≤200	153 (85.47%)	54 (81.82%)	41 (85.42%)	
Triglyceride (mg/dL)**				0.0150
>160	27 (15.08%)	18 (27.27%)	15 (31.25%)	
≤160	152 (84.92%)	48 (72.73%)	33 (68.75%)	

*P value for age comparison was determined by the Kruskal-Wallis test; variables other than age were determined by the chi-square test and Fisher's exact test.

[†]11 patients (6 fat content < 5%, 1 fat level 5%-33%, 4 fat level >33%) had no smoking data and were not included.

[‡]1 patient (fat content <5%) had no data of alcoholic history and was not included. Criteria for alcoholism: alcohol consumption higher than 30 g/day for male patients and 25 g/day for female patients.

[§]1 patient (fat content <5%) had no cirrhosis data and was not included.

^{||}10 patients (6 fat content < 5%, 4 fat content >33%) had no BMI data and were not included.

[¶]10 patients (6 fat content < 5%, 4 fat content >33%) had no hypertension data and were not included.

[#]11 patients (6 fat content <5%, 1 fat level 5%-33%, and 4 fat level >33%) had no data of DM history and were not included.

**1 patient (fat content <5%) had no sufficient serum sample for cholesterol and triglyceride examination and was not included.

High BMI was significantly associated with SH-HCC only in patients with HCV-HCC. Alcoholism was not associated with SH-HCC in any of the patient groups.

COMPARISON OF AGE- AND SEX-MATCHED PATIENTS WITH HBV-HCC, HCV-HCC, OR NBNC-HCC

Because high viral titers may affect hepatocarcinogenesis, we divided patients into high and low viral

titer groups to examine viral titer distribution. High HBV DNA and HCV RNA levels were defined as $\geq 2 \times 10^4$ IU/mL and $\geq 2.3 \times 10^4$ IU/mL, respectively.⁽²⁶⁾ No significant differences were observed in the viral titer distribution between the entire group of patients (2,153) with HBV-HCC and the selected 420 patients with HBV-HCC ($P = 0.9161$) or between the entire group of patients with HCV-HCC and the selected 420 patients with HCV-HCC ($P = 0.2481$) (Supporting Table S6).

TABLE 2. COMPARISON OF THE CLINICAL FEATURES AMONG 1,251 AGE- AND SEX-MATCHED PATIENTS WITH HCC

Variables	HCC Patient Types			P_{HBV}^*		P_{HCV}^*	
	HBV	HCV	NBNC	Uni.	Multi.	Uni.	Multi.
Total patients	420 (33.57%)	420 (33.57%)	411 (32.85%)				
Age				0.3240	0.3000	0.5480	0.5530
Median (range)	66 (27-86)	66 (41-87)	66 (13-88)				
Sex				0.9310	0.7120	0.9310	0.8990
Male	336 (80%)	336 (80%)	327 (79.56%)				
Female	84 (20%)	84 (20%)	84 (20.44%)				
Smoking [†]				0.3610	0.8700	0.5260	0.8920
Yes/Ever	213 (51.57%)	215 (52.57%)	219 (54.89%)				
No	200 (48.43%)	194 (47.43%)	180 (45.11%)				
Alcoholic history [‡]				0.0230	0.1210	0.0140	0.3610
Yes	97 (23.15%)	95 (22.67%)	124 (30.39%)				
No	322 (76.85%)	324 (77.33%)	284 (69.61%)				
Cirrhosis [§]				0.0010	0.0000	0.0000	0.0000
Yes	159 (37.86%)	215 (51.19%)	101 (26.93%)				
No	261 (62.14%)	205 (48.81%)	274 (73.07%)				
Fatty liver (by echography)				0.0030	0.0430	0.0000	0.0190
Yes	62 (14.76%)	50 (11.9%)	95 (23.11%)				
No	358 (85.24%)	370 (88.1%)	316 (76.89%)				
Liver tissue fat content (%)				0.0000	0.0560	0.0000	0.0030
≥33%	23 (5.49%)	19 (4.55%)	48 (16.33%)				
5%-33%	97 (23.15%)	94 (22.49%)	66 (22.45%)				
<5%	299 (71.36%)	305 (72.97%)	180 (61.22%)				
BMI level [¶]				0.0680	0.4560	0.0490	0.4220
Median (range)	23.5 (15.0-37.3)	23.5 (14.0-35.3)	24.1 (15.7-42.7)				
>24	186 (45.04%)	182 (44.61%)	206 (51.63%)				
≤24	227 (54.96%)	226 (55.39%)	193 (48.37%)				
Hypertension ^{¶¶}				0.0080	0.2920	1.0000	0.3430
Yes	89 (21.55%)	122 (29.9%)	119 (29.82%)				
No	324 (78.45%)	286 (70.1%)	280 (70.18%)				
DM history [#]				0.0000	0.0300	0.0440	0.0420
Yes	83 (20.15%)	107 (26.29%)	131 (33%)				
No	329 (79.85%)	300 (73.71%)	266 (67%)				
Cholesterol (mg/dL)**				1.0000	0.5320	0.0000	0.0520
>200	60 (14.29%)	25 (5.95%)	59 (14.39%)				
≤200	360 (85.71%)	395 (94.05%)	351 (85.61%)				
Triglyceride (mg/dL) ^{††}				0.0000	0.0000	0.0000	0.0000
>160	24 (5.73%)	24 (5.73%)	70 (17.07%)				
≤160	395 (94.27%)	395 (94.27%)	340 (82.93%)				

[†]30 patients (7 HBV, 11 HCV, 12 NBNC) had no available smoking data and were not included.

[‡]5 patients (1 HBV, 1 HCV, 3 NBNC) had no available alcoholic history data and were not included.

[§]36 patients with NBNC had no cirrhosis data and were not included.

^{||}In matched patients with HCC, only 1,131 patients (419 HBV, 418 HCV, 294 NBNC) had paraffin tissue blocks for evaluation of fatty change of tissue.

[¶]31 patients (7 HBV, 12 HCV, 12 NBNC) had no BMI and hypertension data and were not included.

[#]35 patients (8 HBV, 13 HCV, 14 NBNC) had no data of DM history and were not included.

**1 patient with NBNC had no sufficient serum sample for cholesterol and triglyceride examination and was not included.

^{††}Two patients (1HBV, 1HCV) had no serum sample for triglyceride examination and was not included.

Abbreviations: Multi, multivariate analysis; P_{HBV} , P values between NBNC and HBV; P_{HCV} , P values between NBNC and HCV; Uni, univariate analysis.

A detailed comparison of all clinical features among the 411 patients with NBNC-HCC, 420 matched patients with HBV-HCC, and 420 matched patients with HCV-HCC is shown in Table 2.

Univariate analysis indicated that most of the metabolic risk factors, including alcoholism, fatty liver (by echography), fatty change of tissue, DM history, and

high TG level (>160 mg/dL), were significantly more associated with patients with NBNC-HCC than with matched patients with HBV-HCC or HCV-HCC. After multivariate analysis, only fatty liver (by echography), fatty change of liver tissue, DM history, and high TG level (>160 mg/dL) remained significantly associated with NBNC-HCC.

TABLE 3. CLINICAL FEATURES OF 399 PATIENTS WITH NBNC-HCC GROUPED BY ALCOHOLIC HISTORY

Variables	Alcoholic history		P
	Yes	No	
Total patients*	124 (30.39%)	284 (69.61%)	
Age (years)			0.1980
Median (range)	62 (28-86)	69 (13-88)	
≤30	1 (0.81%)	4 (1.41%)	
31-40	3 (2.42%)	7 (2.46%)	
41-50	14 (11.29%)	25 (8.8%)	
51-60	41 (33.06%)	48 (16.9%)	
61-70	37 (29.84%)	67 (23.59%)	
>71	28 (22.58%)	133 (46.83%)	
Sex			0.0000
Male	121 (97.58%)	203 (71.48%)	
Female	3 (2.42%)	81 (28.52%)	
Smoking [†]			0.0000
Yes/Ever	107 (86.29%)	110 (40.44%)	
No	17 (13.71%)	162 (59.56%)	
Cirrhosis [‡]			0.0030
Yes	41 (37.61%)	58 (22.05%)	
No	68 (62.39%)	205 (77.95%)	
Fatty liver (by echography)			0.7030
Yes	27 (21.77%)	68 (23.94%)	
No	97 (78.23%)	216 (76.06%)	
BMI level [§]			1.0000
Median (range)	24.1 (15.8-40.0)	24.1 (16.2-42.7)	
>24	64 (51.61%)	140 (51.47%)	
≤24	60 (48.39%)	132 (48.53%)	
Hypertension [§]			0.1930
Yes	31 (25.00%)	86 (31.62%)	
No	93 (75.00%)	186 (68.38%)	
DM history			0.1340
Yes	34 (27.42%)	96 (35.66%)	
No	90 (72.58%)	174 (64.44%)	
Cholesterol (mg/dL) [¶]			1.0000
>200	18 (14.63%)	41 (14.44%)	
≤200	105 (85.37%)	243 (85.56%)	
Triglyceride (mg/dL) [¶]			0.3950
>160	18 (14.63%)	52 (18.31%)	
≤160	105 (85.37%)	232 (81.69%)	

*3 NBNC patients had no alcoholic history data and were not included.

[†]12 patients without alcoholic history had no smoking data and were not included.

[‡]36 patients (15 with alcoholic history and 21 without alcoholic history) had no cirrhosis data and were not included.

[§]12 patients without alcoholic history had no data about BMI and hypertension and were not included.

^{||}14 patients without alcoholic history had no DM history data and were not included.

[¶]1 patient with alcohol drinking history had no data about cholesterol and triglyceride was not included.

We further compared these metabolic risk factors among the 3,543 patients with HCC (excluding the HBV+HCV patients with HCC) and found similar results. The NBNC-patients with HCC differed significantly from patients with HBV-HCC or HCV-HCC in the prevalence of alcoholism, fatty liver

(by echography), high BMI (>24), and DM history by univariate analyses (Supporting Table S7).

COMPARISON BETWEEN PATIENTS WITH NBNC-HCC WITH AND WITHOUT ALCOHOLISM

Three patients with NBNC-HCC with no alcohol consumption data were excluded from the analysis. Male sex, smoking history, and cirrhotic rate were found to be significantly associated with alcoholism (Table 3). No significant differences were found in the metabolic risk factor indices between the patients with NBNC-HCC with or without alcoholism.

SORTING PATIENTS WITH NBNC-HCC AND MATCHED PATIENTS WITH HBV-HCC OR HCV-HCC BASED ON ALCOHOLISM AND CIRRHOSIS FOR FURTHER COMPARISON

We divided the NBNC-HCC, matched HBV-HCC, and matched HCV-HCC patient groups into four groups: 1) nonalcoholic-noncirrhotic, 2) nonalcoholic-cirrhotic, 3) alcoholic-noncirrhotic, and 4) alcoholic-cirrhotic (Fig. 1). The incidence levels of alcoholism and cirrhosis differed significantly between the whole group of 2,153 patients with HBV-HCC and the matched 420 patients with HBV-HCC ($P = 0.0150$). This difference may be due to the significant variation in the age distribution between the 2,153 patients with HBV-HCC and the 411 patients with NBNC-HCC ($P = 0.0000$).⁽²⁶⁾ In contrast, the incidence levels of alcoholism and cirrhosis did not differ significantly between the entire HCV-HCC patient cohort and the selected 420 patients with HCV-HCC ($P = 0.5460$).

Univariate and multivariate analyses of the four groups showed that fatty liver (by echography), high TG levels (>160 mg/dL), and DM history remained significantly associated with NBNC-HCC in the nonalcoholic-noncirrhotic group (Table 4). For the nonalcoholic-cirrhotic groups, only TG levels were significantly higher than those of patients with HCV-HCC. For the alcoholic-cirrhotic and alcoholic-noncirrhotic groups, no significant difference was identified in the incidence of metabolic risk factors between patients with NBNC-HCC and those

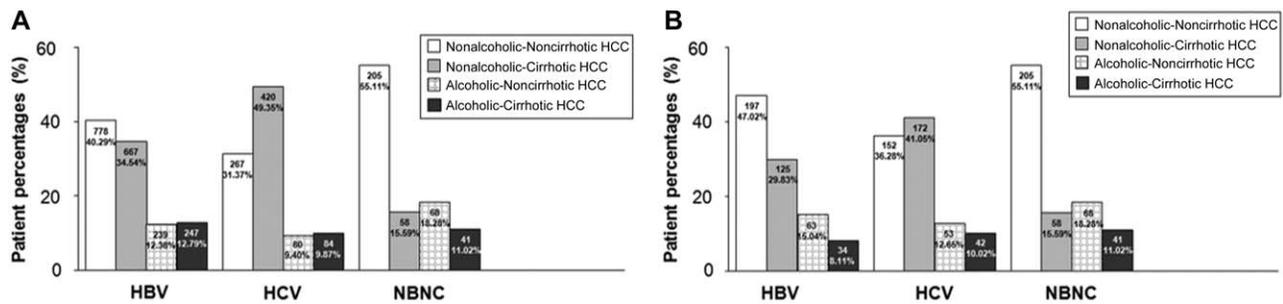


FIG. 1. The distribution of the HCC patients after dividing into 4 groups according to the alcoholic history and cirrhotic status. (A) All 3,533 HCC patient including all HBV-HCC, HCV-HCC and NBNC-HCC patients. (B) The 1,251 HCC patients including all NBNC-HCC patients and matched HBV-HCC, HCV-HCC patients.

matched for HBV- and HCV-HCC (Supporting Tables S8-S10).

TREND ANALYSES FOR ECHOGRAPHY-DETECTED FATTY LIVER, HIGH TG LEVEL, AND DM HISTORY AMONG PATIENTS WITH NBNC-HCC AND MATCHED HBV-HCC OR HCV-HCC

Fatty liver by echography, TG level >160 mg/dL, and DM history were repeatedly shown to be significantly associated with patients with NBNC-HCC compared with matched patients with HBV-HCC or HCV-HCC; therefore, we selected these three risk factors for trend analyses to determine if additive effects could be identified. Patients who were normal for all three risk factors were rated 0, patients who were abnormal for one risk factor were rated 1, and patients who were abnormal for two or three risk factors were rated 2. We found that greater numbers of risk factors involved in analysis resulted in a higher proportion of patients with NBNC-HCC (Cochran-Armitage test, $P = 0.0000$) (Fig. 2A). The same trend analyses were performed on the four groups of patients with HCC based on alcoholic history and cirrhotic status, and all but the alcoholic-cirrhotic group showed that an increasing number of risk factors resulted in a significantly higher proportion of patients with NBNC-HCC compared to the matched patients for HBV-HCC and HCV-HCC (Fig. 2B-E). When we further divided the patients with HCC according to sex, the trends remained for the nonalcoholic-

noncirrhotic group (Fig. 3A,B). However, for female patients in the nonalcoholic-cirrhotic group, the difference among HBV-HCC, HCV-HCC, and NBNC-HCC was not significant (Fig. 3C,D).

Discussion

In our viral hepatitis marker study of 3,843 patients with HCC from the TLCN, the proportion of patients with NBNC-HCC was 10.69%.⁽²⁶⁾ Similarly, in Japan, an HCV-endemic area, the proportion of NBNC-HCC was 6.8%-10% before 2000, but by 2009, this had increased to 17.3%-19%.⁽²⁷⁻³²⁾ A retrospective study by Tateishi et al.⁽²⁸⁾ revealed that the proportion of patients with HCC with nonviral etiologies increased from 10.0% in 1991 to 24.1% in 2010. The incidence in Korea, an HBV-endemic country, was similar (523/4,690, 11.15%).⁽³³⁾ These reports suggest that nonviral HCC is becoming a significant subgroup of HCC in areas of East Asia that were previously endemic for chronic hepatitis C or chronic hepatitis B and have a high incidence of viral-associated HCC.

Most reports on patients with NBNC-HCC in East Asia have been retrospective reviews and only assumed that NAFLD, alcoholism, and DM might be significant risk factors for HCC.⁽²³⁻²⁶⁾ A limitation among these studies of nonviral HCC is that few studies stratified the patients by underlying etiology; this is essential because the etiology of the underlying liver disease could be an important codeterminant in the clinical disease course and the underlying molecular pathogenesis. Nagaoki et al.,⁽³⁰⁾ who included 1,374 patients with HCC in their study (from 1995 to 2009),

TABLE 4. COMPARISON OF CLINICAL FEATURES AMONG 554 PATIENTS WITH NONALCOHOLIC NONCIRRHOTIC HCC

Variables	HCC Patient Types			P_{HBV}^*		P_{HCV}^*	
	HBV	HCV	NBNC	Uni.	Multi.	Uni.	Multi.
Total patients	197 (35.56%)	152 (27.44%)	205 (37%)				
Age				0.0430	0.3490	0.8400	0.6280
Median (range)	66 (27-85)	69 (44-87)	70 (13-88)				
Sex				0.1670	0.0970	0.0820	0.5000
Male	154 (78.17%)	122 (80.26%)	147 (71.71%)				
Female	43 (21.83%)	30 (19.74%)	58 (28.29%)				
Smoking [†]				0.8350	0.4220	0.5070	0.5630
Yes/Ever	75 (39.47%)	66 (44.9%)	79 (40.72%)				
No	115 (60.53%)	81 (55.1%)	115 (59.28%)				
Fatty liver (by sonography)				0.0030	0.0330	0.0030	0.0410
Yes	27 (13.71%)	20 (13.16%)	53 (25.85%)				
No	170 (86.29%)	132 (86.84%)	152 (74.15%)				
Fat content (%) in nontumor liver tissue [‡]				0.0090	0.6750	0.0030	0.5010
≥33%	11 (5.58%)	5 (3.31%)	24 (13.95%)				
5%-33%	35 (17.77%)	33 (21.85%)	37 (21.51%)				
<5%	151 (76.65%)	113 (74.83%)	111 (64.53%)				
BMI level [§]				0.2190	0.4980	0.3800	0.6550
Median (range)	23.1 (15-35.8)	23.2 (14.5-35.1)	23.6 (16.2-42.7)				
>24	78 (41.05%)	62 (42.47%)	92 (47.42%)				
≤24	112 (58.95%)	84 (57.53%)	102 (52.58%)				
Hypertension				0.0500	0.0750	1.0000	0.5750
Yes	36 (18.95%)	47 (32.19%)	62 (31.96%)				
No	154 (81.05%)	99 (67.81%)	132 (68.04%)				
DM history [¶]				0.0020	0.1610	0.0010	0.0030
Yes	36 (19.05%)	24 (16.55%)	64 (33.33%)				
No	153 (80.95%)	121 (83.45%)	128 (66.67%)				
Cholesterol (mg/dL)				0.7800	0.8640	0.0150	0.0460
>200	30 (15.23%)	9 (5.92%)	29 (14.15%)				
≤200	167 (84.77%)	143 (94.08%)	176 (85.85%)				
Triglyceride (mg/dL) [#]				0.0000	0.0040	0.0010	0.0260
>160	12 (6.09%)	10 (6.62%)	38 (18.54%)				
≤160	185 (93.91%)	141 (93.38%)	167 (81.46%)				

[†]23 patients (7 HBV; 5 HCV; 11 NBNC) had no available smoking data and were not included.

[‡]520 patients (197 HBV, 151 HCV, 172 NBNC) had available paraffin tissue blocks for evaluation of tissue fatty change.

[§]24 patients (7 HBV, 6 HCV, 11 NBNC) had no BMI data and were not included.

^{||}24 patients (7 HBV, 6 HCV, 11 NBNC) had no hypertension data and were not included.

[¶]28 patients (8 HBV, 7 HCV, 13 for NBNC) had no data of DM history and were not included.

[#]1 HCV patient had no serum sample for triglyceride examination and was not included.

Abbreviations: Multi, multivariate analysis; P_{HBV}^* , P values between NBNC and HBV; P_{HCV}^* , P values between NBNC and HCV; Uni, univariate analysis.

demonstrated that the incidence of DM, heavy alcohol consumption, hypertension, and hyperlipidemia in 209 patients with NBNC-HCC was significantly higher than among patients with HBV-HCC or HCV-HCC. In the present study, we also stratified patients based on their viral hepatitis status, but our results differed from those of Nagaoki et al. In our study, multivariate analyses showed that only fatty liver by echography, DM history, and high serum TG level were significantly associated with NBNC-HCC, whereas alcoholism and hypertension were not. Than et al.⁽⁴⁾ and Kaibori et al.⁽³¹⁾ also reported that excessive alcohol consumption was not significantly

different between NBNC-HCC and HBV- or HCV-related HCC.

Among the 3,843 patients with HCC, the proportion of patients with HCV-HCC without cirrhosis was 40.75%. For the matched 1,251 patients with HCC, the proportion of HCV-HCC without cirrhosis was 48.81% and the age range was 41-87 years. This wide age range and high proportion of noncirrhosis differ from published reports.^(4,39) One explanation is that the majority (about 90%) of patients recruited in the TLCN were operable patients so that we could collect tumor tissue samples. Therefore, many of the patients with HCC and cirrhosis may not have been

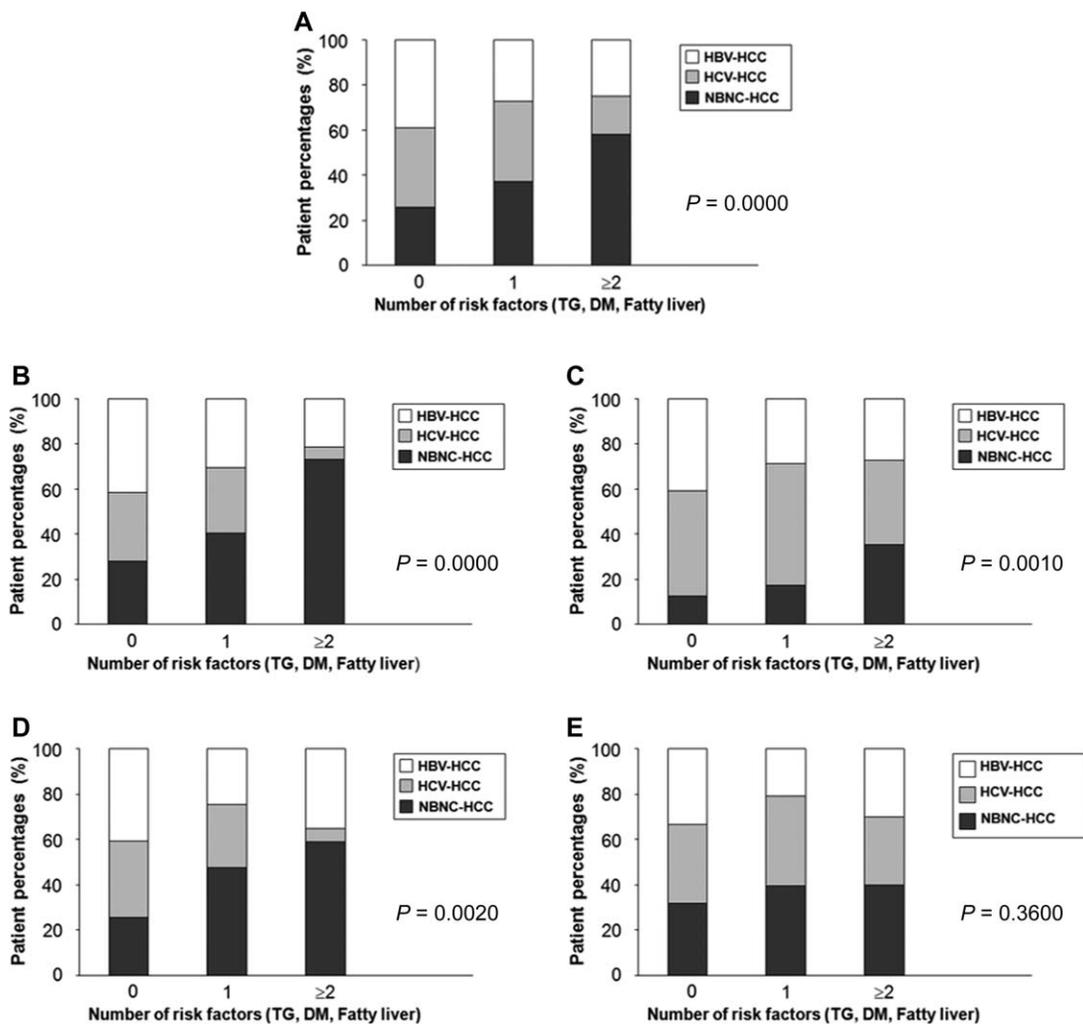


FIG. 2. Trend analyses for three risk factors, fatty liver by echography, TG level >160 mg/dL, and DM history, among 1,251 patients with HCC, which includes all patients with NBNC-HCC and the matched patients with HBV-HCC or HCV-HCC. Patients who were normal for all three risk factors are scored as 0, patients who were abnormal for one risk factor are scored as 1, and patients who were abnormal for two or three risk factors are scored as 2. (A) All patients with NBNC-HCC and matched patients with HBV-HCC or HCV-HCC (n = 1,251). (B) Nonalcoholic-noncirrhotic group (n = 554). (C) Nonalcoholic-cirrhotic group (n = 355). (D) Alcoholic-noncirrhotic group (n = 184). (E) Alcoholic-cirrhotic group (n = 117). All four groups except the alcoholic-cirrhotic group showed that as the number of risk factors increased so did a significant proportion of patients with NBNC-HCC compared with the matched patients with HBV-HCC or HCV-HCC.

recruited in the TLCN. The second reason could be due to ethnic or genetic factors. Because Taiwan is an endemic area of HBV, the possibility that occult HBV infection plays a role may also need to be considered.

When the patients with NBNC-HCC were divided into alcoholic and nonalcoholic groups, we found no significant difference in the association with metabolic risk factors. Additionally, no significant differences were found between the patients with NBNC-HCC and alcoholism and the matched patients with HBV-HCC or HCV-HCC who also had a history of

alcoholism. Alcoholism is known to be associated with a higher risk for metabolic syndromes.⁽⁴⁰⁾ Our data suggest that this association is not affected by co-existing chronic viral hepatitis.

In patients with cirrhosis with or without alcoholism, fatty change of liver tissue was the only factor that was significantly associated with NBNC-HCC by univariate and multivariate analyses. Based on the histological examination, 27.5% and 28.57% of patients with NBNC-HCC and cirrhosis without and with alcoholism, respectively, exhibited a high degree of

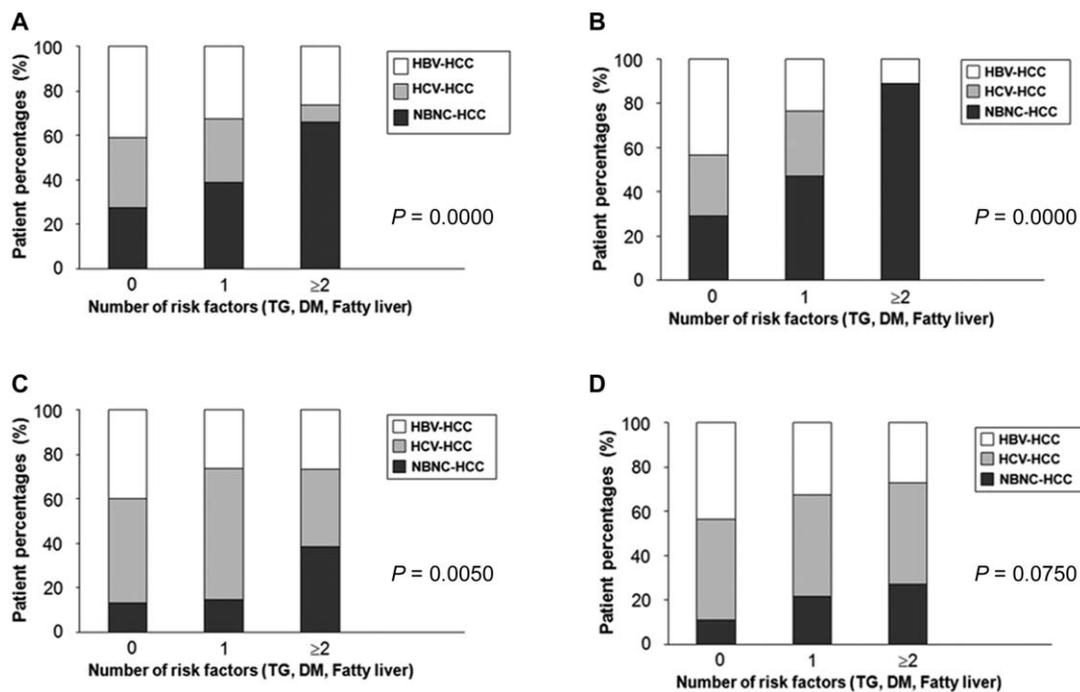


FIG. 3. Trend analyses of the difference between sexes for three risk factors, fatty liver by echography, TG level >160 mg/dL, and DM history, in patients with HCC. (A) Nonalcoholic-noncirrhotic male patients ($n = 423$). (B) Nonalcoholic-noncirrhotic female patients ($n = 131$). (C) Nonalcoholic-cirrhotic male patients ($n = 248$). (D) Nonalcoholic-cirrhotic female patients ($n = 107$). Only female patients with HCC and cirrhosis with no alcoholism showed no significant difference when compared with matched patients with HBV-HCC or HCV-HCC.

fatty change in tissue ($\geq 33\%$). In contrast, among the matched patients with HBV-HCC or HCV-HCC, only up to 5.6% of patients with cirrhosis without alcoholism and up to 4.88% of patients with cirrhosis and alcoholism exhibited a high degree of fatty change to tissue ($\geq 33\%$). These results suggest that fatty change in tissue may be pathognomonic and play some role in the hepatocarcinogenesis of nonviral HCC. In their study of the mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease, Mota et al.⁽⁴¹⁾ described the involvement of the cytokine pathway and caspase activation and identified the common denominator as metabolic derangement, such as glucose intolerance and insulin resistance in tissue. The potential link between those cytokine pathways and hepatocarcinogenesis warrants further investigation.

SH-HCC has also been reported to be associated with metabolic syndrome,^(37,38) which we confirmed in this study. However, we only identified DM history to be significantly associated with SH-HCC in the three groups of patients with HCC, while hypertension, high serum CHO, and TG levels were not. Thus, our data differ

slightly from the two published reports.^(37,38) Because SH-HCC has not been found to be associated with prognosis, its clinical significance will need further study.

By using trend analyses, we showed that significantly more patients with NBNC-HCC presented more than two abnormal metabolic risk factors compared to patients in other HCC groups.

Among the four groups of patients classified on the basis of alcoholic history and cirrhotic status, the nonalcoholic-noncirrhotic group of patients with NBNC-HCC exhibited the greatest difference from the matched patients with HBV-HCC or HCV-HCC ($P = 0.0000$).

This strongly suggests that metabolic risk factors play a significant role in hepatocarcinogenesis. The nonalcoholic-cirrhotic group exhibited less of a difference, although still significant, with matched HBV- and HCV-HCC groups ($P = 0.0010$).

These results are reasonable because the etiology of cirrhosis in these patients may have included other etiologies, such as autoimmune hepatitis, primary biliary cirrhosis, or genetic abnormality.

Occult hepatitis viral infection may also require consideration. In the current study, the patients with NBNC-HCC were negative for HBsAg, HBV DNA, and anti-HCV antibody. We did not include the data of hepatitis B core antibodies (anti-HBc) in these analyses because related data were not included in the medical records of the majority of patients with HCC.

However, Taiwan is an endemic area for HBV infection, and the prevalence of anti-HBc may be high for people born before universal vaccination was instituted (>30 years). Further analyses on the status of anti-HBc, hepatitis B surface antibody, and tissue HBV DNA in the 411 patients with NBNC-HCC will follow to clarify the role of occult HBV infection in these patients.

We were able to perform a detailed clinical analysis of a large number of patients with HCC mainly because of the success of the TLCN in recruiting patients with HCC and building a comprehensive clinical database, which includes epidemiology data based on detailed questionnaires. In addition, the TLCN's liver tissue bank is large, and this enabled us to perform a histopathological review of relevant HCC tissues. We were also able to use serum samples from the tissue bank to perform retrospective marker studies for metabolic syndrome.

In conclusion, we demonstrated that the clinical characteristics of patients with NBNC-HCC differ from those of patients with HBV-HCC or HCV-HCC. Metabolic risk factors, such as fatty liver (by echography), high TG levels (>160 mg/dL), and DM history, were significantly associated with patients with NBNC-HCC, especially those without alcoholism and cirrhosis. Because of the success of universal vaccination and antiviral therapy in Taiwan and East Asia, viral HCC is decreasing. Therefore, strategies for cancer prevention, prediction, and surveillance for HCC will require modification.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1182/full.